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Effects of Oxytocin Administration on Oxidative Markers in the Temporal Lobe of Aged Rats

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Considering the relevance of oxidative stress in aging and antioxidant properties of oxytocin, which we previously demonstrated, we studied the effects of intraperitoneal oxytocin administration (10 mg/kg body mass for 12 days) on enzymatic activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx), and also on malonic dialdehyde (MDA) level in the brain temporal lobe of aged (2-year-old) Wistar rats. In these rats, oxytocin treatment provided a significantly lower SOD enzymatic activity, somewhat greater GPx activity, and significantly lower MDA concentration, when compared to the respective indices in aged control rats. It is concluded that, due to its antioxidant effect, oxytocin can be considered a candidate for the development of a new therapeutic modality in aging and in related neuropsychiatric disorders.

Keywords: aging, oxytocin, oxidative stress, superoxide dismutase (SOD), glutathione peroxidase (GPx), malonic dialdehide (MDA), temporal lobe, rat.

INTRODUCTION

In mammals, along with canonical roles in parturition and lactation [1], oxytocin exerts pleiotropic effects on the brain and other organs. Oxytocin contributes to the modulation of sexual excitement, emotional behavior, and other forms of behavior. This neuropeptide promotes confidence and demonstrates a tranquilizing effect due to its capacity to diminish anxiety and feelings of fear. Nowadays, there is rising interest in understanding modulation of higher nervous activity by oxytocin; as is believed, it can be used as a possible therapeutic agent in some neuropsychiatric disorders, such as autism [2, 3], schizophrenia [4], anxiety [5], depression [6], or some types of dementia [7, 8]. In addition, there are ongoing studies suggesting that disorders in oxytocin-related processes could also contribute to the pathogenesis of aging-related diseases, Alzheimer's disease in particular [9]. The latter is the most important and most widespread neurodegenerative disorder, as judged by the number of patients affected worldwide. It is estimated that nearly 7% of the population at age above 65 years are affected by Alzheimer's disease [10].

When it comes to the main theories related to the aging processes, the exact causes are poorly understood [11]. A majority of authors suggest aging as a multifactorial process [12] with oxidative stress being an important factor [13–15]. At the same time, there are controversies in the literature regarding the association that might exist between activity of the oxytocinergic system and aging, with experimental and clinical reports describing blood oxytocin levels in the aged individuals as decreased [16–18], unchanged [19–22], or even increased in some cases [23]. In this respect, our group has demonstrated earlier a progressively increasing oxidative state in human patients ranging from mild cognitive impairment (an intermediate state between physiological aging and dementia) to clear Alzheimer's disease [24].

Taking into account the relevance of oxidative stress to aging, as well as the fact that oxidative stress can be weakened by some manipulations, a lot of antioxidant medications were used as anti-aging agents, such as synthetic products [25] and plant extracts [26–28] (some of them with encouraging results). Previously, we have confirmed

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the antioxidant effects of oxytocin administration in Wistar rats [29], while other groups also have shown in animal research that oxytocin injections protect various tissues against oxidative impairment [30–33].

The latest findings in this field of research are rather encouraging. In the communication by Elabd et al. published in *Nature* [34], impressive reparatory properties of oxytocin in aged individuals regarding muscle maintenance and regeneration were demonstrated. As reported in another paper [35], just a single intranasal dose of oxytocin could significantly improve social cognition and related behavioral manifestations in a frontal temporal dementia pathology. Oxytocin administration in rats could alleviate some aged-associated deficits by down-regulating proinflammatory cytokines, such as IL-6, TNF-α, and IL-1β [36], which might be explained by the well-known correlation between oxidative stress and inflammation [37].

In this our study, we evaluated the effectiveness of systemic administration of oxytocin with regard to oxidative stress in the brain temporal lobe (the cortex area that is most sensitive to oxidative damage) of aged Wistar rats [38]. Superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities and also malonic dialdehyde (MDA) levels were measured in the brain tissues.

METHODS

Animal Housing and Habituation. The animals were housed in woodchip-bedded polyacrylic cages (5 animals per cage). They were maintained in a temperature- and environment-controlled room (22 ± 2°C, a 12/12-h photoperiod cycle, and relative humidity of 40–60%). Standard food and water were available *ad libitum*.

Treatments. Aged (two-year-old) male Wistar rats weighing 270–300 g were randomly allocated to two groups (n = 5 per group). The oxytocin group (group Age+Ox) received i.p. injections of oxytocin (Sigma-Aldrich LLC., Germany) in a daily dose of 10 mg/kg body mass for 12 consecutive days, while control animals (group Age) were injected with saline.

Tissue Collection. After the last treatment day, the rats were anesthetized and rapidly decapitated. Their brains were removed, and the temporal lobes were collected. Each temporal tissue sample was weighed and homogenized with a Potter homogenizer coupled with a Cole Parmer Servodyne mixer in bidistilled water (1.0 g tissue/ 10 ml). Homogenates were centrifuged for 15 min at 3000 rpm; the supernatants were separated in aliquot 1.5 ml Eppendorf tubes and stored at –22°C until biochemical analysis.

Biochemical Assays. The SOD enzymatic activity was measured by an indirect method of competitive inhibition with a commercial assay (SOD Assay Kit, Sigma-Aldrich, USA). The principle of the method is based on the Dojindo's tetrazolium salt (WST-1) reaction with superoxide anion in forming a water-soluble formazan dye. The O_2 reduction rate is linearly related to xanthine oxidase activity, and the inhibition activity can be estimated by colorimetry. Thus, relative inhibition by SOD is an indirect way of determining SOD activity. The assay endpoints were monitored by the absorbance at 450 nm after 20 min of the reaction at 37°C [39]. The measurements were performed according to the manufacturer's instructions. The SOD activity was, therefore, expressed as the inhibition rate (%).

The GPx activity was also quantified by an indirect method of substrate-consumption dynamic observation. The method measures the rate of NADPH consumption (at 340 nm) during the considered time unit (see below) as an index for GPx activity. A GPx Cellular Activity Assay Kit (Sigma, USA) was used, and all the manufacturer's instructions were followed using a Beckman Coulter DU-700 series (Beckman Coulter, Canada) spectrophotometric system. The decrease in the NADPH amount measured at 340 nm during oxidation of NADPH to NADP is indicative of the level of GPx activity [40]. As a result, the latter will be further expressed as GPx enzyme units/ min (U/min). The method is based on oxidation of glutathione (GSH) to oxidized glutathione (GSSG) catalyzed by GPx, which is then coupled with recycling GSSG back to GSH in the presence of glutathione reductase (GR) and NADPH.

Malonic dialdehyde (MDA) levels were determined by the thiobarbituric acid-reacting substances assay. Two hundred microliters of supernatant were added and mixed with 1.0 ml of 50% trichloroacetic acid, 0.9 ml of Tris–HCl (pH 7.4), and 1.0 ml of 0.73% thiobarbituric acid. After vortex mixing, samples were maintained at 100°C for 20 min. Afterwards, samples were centrifuged at 3000 rpm for 10 min, and the supernatant was read at 532 nm in a UV-VIS spectrophotometrical system (Beckman Coulter,

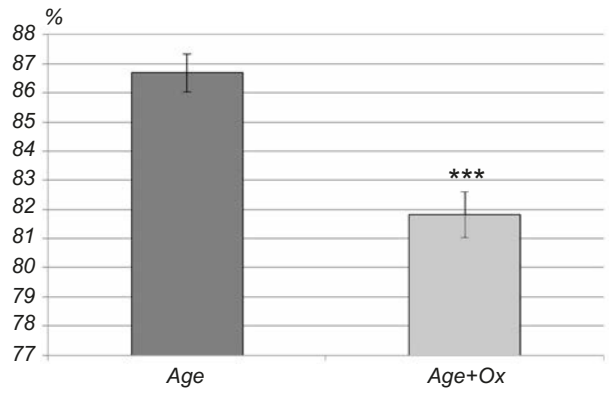
Canada). The signal was read against an MDA standard curve, and the results were expressed as mmol/g of the temporal lobe extract [41].

Statistics. For statistical relevance, all samples were run in duplicate, and the means were considered in statistical analysis. The standard ANOVA test, Pearson coefficient calculation, and HDS honesty test were performed; when the samples followed the normal distribution, the Student *t*-test was used. The results per group were expressed as means ± s.e.m., and the differences between the experimental and control groups were regarded as statistically significant at $P < 0.05$.

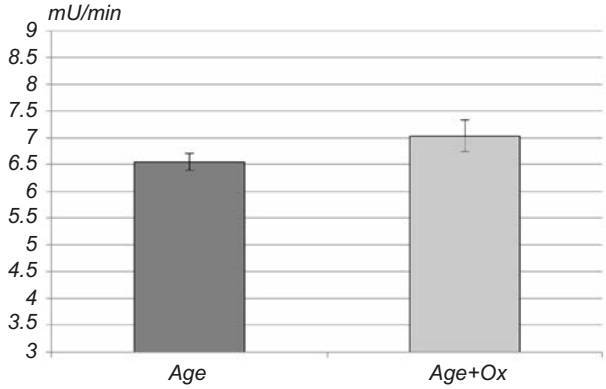
RESULTS

As a result of oxytocin administration, a significant decrease (by 5.7%, on average) of the SOD inhibition rate in the temporal lobe was observed, as compared to the controls (Fig. 1; $P = 0.0015$). However, in the case of another antioxidant enzyme, namely GPx, administration of oxytocin resulted in a slight trend toward increase in the respective values in the temporal lobe tissue, when compared to the control group of aged animals (Fig. 2), although the difference was insignificant ($P = 0.16$).

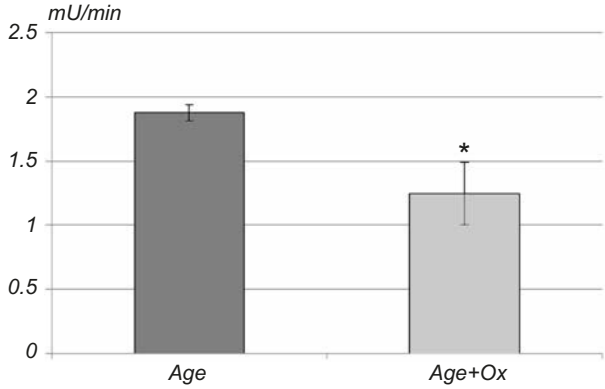
Regarding the concentrations of MDA (the lipid peroxidation marker) in the temporal lobe, we observed an antioxidant action of oxytocin administration. The oxytocin-treated group showed significantly lower MDA tissue levels (67%, on average), as compared to those in saline-treated animals (Fig. 3; $P = 0.025$).



F i g. 1. Effect of oxytocin intraperitoneal (i.p.) administration on superoxide dismutase (SOD) activity in the rat temporal lobe of aged rats. Age and Age+Ox are groups of control aged (2-year-old) rats and similar animals treated with oxytocin. Vertical scale) Inhibition rate of SOD activity, %. Values are means ± ± s.e.m. ($n = 5$ per group). ***$P = 0.0015$ *vs*. aged control.



F i g. 2. Effect of oxytocin i.p. administration on glutathione peroxidase (GPx) activity (mU/min) in the rat temporal lobe. Other designations are the same as in Fig. 1. $P = 0.16$ *vs*. aged control.



F i g. 3. Effect of oxytocin i.p. administration on the malonic dialdehyde (MDA) concentration in the rat temporal lobe. Designations are the same as in Figs. 1 and 2. *$P = 0.025$ *vs*. aged control.

DISCUSSION

Current theories describing the mechanisms underlying the aging process presume the significant roles of free radicals, mitochondrial deficiencies, telomere shortening, DNA damage, protein glycation, and other alterations [12]. In Alzheimer's disease patients, there is a strong correlation between aging and some oxidative stress markers in blood serum. This correlation was demonstrated in large European clinical studies, e.g., in the ZENITH study in 188 patients from France, Italy, and Great Britain [42]. This was also confirmed in individual studies in various groups and populations [13–15]. As was shown in our earlier research, there is a vicious cycle in the plasma of individuals with mild cognitive impairment and, subsequently, of Alzheimer's disease patients. In these subjects, the reduced antioxidant enzymatic system becomes,

at some point, unable to cope with increased production of pro-oxidants [24]. Also, rather similar aspects regarding a relevance of oxidative stress to aging were demonstrated in the reproductive system, with increased levels of oxidative stress markers found in the semen [43, 44] and oocytes; this affected fertilization [45]. Comparative changes were found in the ocular system [46, 47].

Thus, considering participation of free radicals and mitochondrial-related oxidative stress in aging and certain diseases [11, 48, 49], some authors have come to a conclusion that using antioxidant drugs could exert an anti-aging effect because oxidative stress is, at least partly, a correctable factor [11].

It was demonstrated that drugs capable of reversing some age-associated oxidative stress processes could have a noticeable therapeutic potential. For example, it was shown that an experimental pharmaceutical product, MRN-100 (an iron-based hydroferrate fluid), is able to reverse some age-associated oxidative stress manifestations in rats [25]. Other groups reported that some plant extracts, such as that of *Crocus sativus*, reduce oxidative stress markers and the increased pro-inflammatory status in aged rats [26]. Silymarin, a lipophilic fruit extract, reduced lipid peroxidation and protein oxidation in the aged rat brain [27] and exerted protective effects on aging-related and cortical neuropsychiatric manifestations.

As has been mentioned above, the association of oxytocin with aging is still controversial. An increased oxytocin level was reported in the *post-mortem* brains (in particular, in the hippocampus and temporal cortex) of 12 Alzheimer's disease individuals [23]. Other groups showed a decreased number of oxytocinergic neurons in the paraventricular nucleus of aged patients [16] and decreased oxytocinergic responses to stressful situations [18]. On the other hand, there are studies failing to find any modifications of oxytocin in aging, e.g., in the parabrachial nucleus [19]. Other similar studies referred to the paraventicular nucleus [20] or cerebrospinal fluid oxytocin concentrations [21] in Alzheimer's disease patients and aged monkeys [22]. In addition, it was found that oxytocin gene expression is not modified in depressed Alzheimer's patients *vs*. a non-depressed Alzheimer's group [50]. This is an important observation taking into consideration the increasing interest in depression as a possible risk factor for Alzheimer's disease [51]. In this regard, it should be mentioned that, in addition to Alzheimer's disease [24], our group demonstrated a gradual increase of the oxidative stress status in major depression disorder [52]. All these contradictory results regarding oxytocin implication in the aging-related processes could be related to diurnal and seasonal variations, different methods used, a variety of brain areas and biological samples examined, and substantial differences between human patients and animal models [53].

Systemic oxytocin administration compensates a decrease of its blood plasma level in aged individuals and exerts some reparatory effects [34]. However, oxytocin administration (3–6 ng/kg) in 24 to 28-month-old rats resulted in rather negative effects in the social memory and behavioral despair tests [54]. On the other hand, there are studies showing that oxytocin could miligate memory disorders or anxiety-related stress [54–56].

Previous studies suggested that i.p. oxytocin administration for 5 days in a dose of 3 mg/kg could rescue some specific aged-related insulin resistance, possibly through modulation of the expression of main inflammatory genes (e.g., those of IL-1β, IL-6, and TNF-α), as well as by decreasing the intensity of oxidative stress (which is manifested in shifts in the levels of the respective markers, in our case malonic dialdehyde) [36].

In this our study, we demonstrated a possible antioxidant effect of i.p. oxytocin administration; a considerale decrease in the MDA level in the temporal lobe of aged rats was observed. It would be reasonable to study the effects of systemic oxytocin administration on the hippocampus because there is an association of aging-related memory deficits with oxidative stress precisely in this brain area [57, 58].

On the other hand, a concomitant significant decrease of the SOD inhibition rate with mild changes in GPx activity was found in the oxytocin-treated group. This could be a compensatory response to oxytocin-induced suppression of lipid peroxidation, though oxytocin under some conditions can enhance oxidative processes [59]. Moreover, our research group demonstrated earlier (a brief communication and a full-text paper) that some dosages of oxytocin in zebrafish models could decrease specific activities of both SOD and GPx [60, 61]. In this regard, it is reasonable to mention a paper by Sirota et al. [62], in which preconditioned rats demonstrated, after 30- and 360-min exposures to immobilization stress, a low level of brain SOD activity, as compared to the controls (due, perhaps, to stress-induced decrease in the level of reactive oxygen species).

Regarding the limitations of our study, it should be mentioned that we did not explore a young control group. In order to reduce the number of animals for ethical reasons, we considered an overwhelming number of studies that already proved that aging results in a significant intensification of the oxidative stress status.

The positive effects of oxytocin on the MDA concentration in the temporal lobe of aged rats in our study could be partly explained by a decrease of systemic and local background age-related inflammation. It was recently demonstrated both *in vivo* (in mice) and *in vitro* that oxytocin decreases manifestations of LPS-induced inflammation in microglial cells, which is relevant to the majority of neuroinflammatory disorders [63].

We believe that modification of the MDA concentration (an oxidative stress marker) in the temporal lobe in our study was caused by a direct influence of i.p. injected oxytocin on the brain because it overcomes the blood-brain barrier and reaches brain tissues, similarly to what was found at nasal administration of this peptide [64, 65].

Therefore, we observed certain clear antioxidant effects of peripheral oxytocin administration in aged Wistar rats. There were a significant decrease of SOD activity, some increase (insignificant) of GPx activity, and a considerable decrease in the MDA concentration in the temporal brain lobe tissue, when compared to that in aged control rats.

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Rats were treated in accordance with the current national and European regulations regarding the scientific research using vertebral animals, in accordance with the NIH-Care and Use of Laboratory Animals Manual (8th Edition), and the guidelines on animal bioethics from the Animal Experimentation and Animal Health and Welfare Act by Romania. All procedures were in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). Maximum efforts were made to minimize animal suffering and to reduce the number of animals used.

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